

We Claim:

1. A method for microdissecting tissue, comprising:
labeling a sample of the tissue by exposing the tissue to a sufficient
concentration of a fluorescent specific binding agent for a sufficiently short period of
5 time to reduce a binding time of the agent to the tissue to reduce loss of a biological
molecule in the tissue; and
identifying a component of interest in the sample to which the fluorescent
specific binding agent binds by detecting fluorescence of the component of interest in
the tissue; and
10 microdissecting components of interest from the tissue.
2. The method of claim 1, wherein identifying the component comprises
intensifying a signal from the fluorescent specific binding agent to provide an
intensified image signal.
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3. The method of claim 2, wherein microdissecting comprises dissecting tissue
components with a laser beam, and the method further comprises selectively filtering an
image of the laser beam to reduce laser-induced distortion of the intensified image.
- 20 4. The method of claim 1, wherein the specific binding agent comprises an
aqueous solution, and the biomolecule is RNA, DNA or a protein, which is lost in the
presence of water.
5. The method of claim 4, wherein the biomolecule is RNA.
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6. The method of claim 1, wherein microdissecting comprises applying a
capture member to the sample of tissue, and applying laser energy to the component of
interest to adhere the component to the capture member.

7. The method of claim 1, wherein the sufficient concentration of fluorescent specific binding agent is sufficient to avoid loss of more than about 5% of the biomolecule.

5 8. The method of claim 7, wherein the sufficient concentration of fluorescent specific binding agent is sufficient to avoid loss of more than about 10% of the biomolecule.

10 9. The method of claim 1, wherein the fluorescent specific binding agent includes a fluorescent antibody, lectin, protein A, protein G and mixtures thereof.

10 10. The method of claim 1, wherein the sufficient concentration of specific binding agent is at least 0.02 mg/mL

15 11. The method of claim 10 wherein the sufficient concentration of specific binding agent is at least 0.1 mg/mL.

20 12. The method of claim 9, further comprising pre-mixing a primary antibody and a secondary antibody, at least one of which is fluorescent, prior to exposing the tissue to the specific binding agent to reduce a time of exposure of the tissue to the specific binding agent.

25 13. The method of claim 2, wherein the fluorescent specific binding agent is present in a sufficient concentration that, when the tissue is exposed to the fluorescent specific binding agent for less than about 5 minutes, the intensified image signal is detectable.

14. The method of claim 13, wherein the fluorescent specific binding agent is present in a sufficient concentration that, when the tissue is exposed to the fluorescent

specific binding agent for less than about 3 minutes, the intensified image signal is detectable.

15. The method of claim 14, wherein the fluorescent specific binding agent is present in a sufficient concentration that, when the tissue is exposed to the fluorescent specific binding agent for not more than about 1 minute, the intensified image signal is detectable.

16. The method of claim 3, wherein microdissecting comprises targeting tissue components with a target laser beam, and viewing the intensified image through an infrared filter that selectively minimizes image distortion caused by the laser beam, without eliminating the signal image.

17. A method of performing tissue microdissection of a tissue specimen, comprising:

exposing the tissue specimen to at least one fluorescently labeled antibody which specifically binds to a component of interest in the tissue, wherein the tissue is exposed to a sufficient concentration of the antibody, in an aqueous solution, for a sufficient period of time to label the component of interest without substantially degrading RNA in the tissue;

washing unbound antibody from the tissue;

intensifying an image of the tissue specimen which has been exposed to the fluorescently labeled antibody, to obtain an intensified fluorescent signal from the tissue;

applying a transfer member to the tissue;

directing a target laser beam to the component of interest in the tissue, to mark the component that is to be dissected from the tissue specimen, while viewing the target laser beam through an infrared filter that selectively filters infrared radiation but not the fluorescent signal, to minimize heat distortion of the intensified image, while still viewing the intensified signal; and

applying radiant laser energy to the component of interest to transfer the component to the transfer member.

18. The method of claim 17, wherein exposing the tissue to a sufficient
5 concentration of the at least one fluorescently labeled antibody comprises exposing the tissue to a concentration of at least 0.04 mg/mL of the fluorescently labeled antibody.

19. The method of claim 18, wherein exposing the tissue to a sufficient
10 concentration of the at least one fluorescently labeled antibody comprises exposing the tissue to a concentration of at least 0.10 mg/mL of the at least one fluorescently labeled antibody

20. The method of claim 17, wherein exposing the tissue to the at least one
15 fluorescently labeled antibody comprises exposing the tissue to the at least one fluorescently labeled antibody for less than about 5 minutes.

21. The method of claim 20, wherein ex exposing the tissue to the at least
one fluorescently labeled antibody comprises exposing the tissue to the at least one fluorescently labeled antibody for less than about 3 minutes.

22. The method of claim 21, wherein ex exposing the tissue to the
20 fluorescently labeled antibody comprises exposing the tissue to the antibody for no more than about 1 minute.

23. An apparatus for rapid immunofluorescence laser capture microdissection,
25 comprising:

a laser capture microdissection microscope;

a light source capable of exciting fluorescence from fluorescent specific binding agents disposed to illuminate a sample placed on the sample stage of the microscope;

an image intensifier disposed between the sample stage of the microscope and
an image acquisition system; and

an infrared filter disposed between the sample stage and the image intensifier.

5 24. A method for fluorescently staining a tissue section for microdissection,
comprising:

fixing a tissue section with a non-crosslinking fixative;

10 rinsing the tissue section twice with an aqueous buffered solution for about 5
seconds per rinse;

incubating the fixed tissue section with an aqueous fluorescent specific binding
agent solution of sufficient concentration to selectively label cells within the tissue
section in about 1 minute.

15 rinsing the tissue section twice with an aqueous buffered solution for about 5
seconds per rinse;

dehydrating the tissue section; and

drying the tissue section.

25 25. The method of claim 24, wherein the aqueous buffered solution is
20 diethylpyrocarbonate-treated phosphate-buffered saline solution.

26. The method of claim 24 wherein the fluorescent specific binding agent
solution comprises a primary antibody covalently linked to a fluorescent molecule.

25 27. The method of claim 24, wherein the fluorescent specific binding agent
solution comprises a pre-mixed solution of primary antibody and fluorescently labeled
secondary antibody.

28. The method of claim 24, wherein the fluorescent specific binding agent solution comprises a premixed solution of a fluorescently labeled primary antibody and a fluorescently labeled secondary antibody.

5 29. The method of claim 24, wherein the fluorescent specific binding agent solution comprises a solution of fluorescently labeled lectin.

30. The method of claim 24, wherein the fluorescent specific binding agent solution comprises a mixture of primary antibody and fluorescently labeled protein A or
10 G.

31. The method of claim 30, wherein primary antibody is also fluorescently labeled.

15 32. The method of claim 24, wherein the non-crosslinking fixative is selected from the group consisting of ethanol, acetone, methanol and mixtures thereof.

33. The method of claim 24, wherein the fluorescent specific binding agent solution further comprises an enzyme inhibitor.

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34. The method of claim 33, wherein the enzyme inhibitor is chosen from the group consisting of RNase inhibitor, DNase inhibitors, protease inhibitors and mixtures thereof.

25 ³⁵ 34. A method for immunofluorescently labeling tissue that preserves biological molecules, comprising:

contacting the tissue with aqueous fluorescent antibody solutions of sufficient concentration to selectively label immunophenotypically similar cells against which the fluorescent antibodies are directed in less than about five minutes.

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Author	Year	Country	Sample Size	Method	Findings
Smith et al.	2001	USA	1,200	Survey	High levels of stress and anxiety reported among adolescents.
Johnson et al.	2003	UK	800	Interview	Adolescents with mental health problems often experience social isolation.
Lee et al.	2005	Canada	1,500	Survey	Increased risk of substance use among adolescents with mental health issues.
Kim et al.	2007	South Korea	900	Survey	Family support significantly impacts adolescent mental health outcomes.
White et al.	2009	Australia	1,100	Survey	Adolescents with mental health problems often experience stigma.
Green et al.	2011	USA	1,300	Survey	Access to mental health services is a major barrier for many adolescents.
Black et al.	2013	UK	1,000	Survey	Adolescents with mental health problems often experience academic difficulties.
Brown et al.	2015	USA	1,400	Survey	Peer support programs show promise in improving adolescent mental health.
Miller et al.	2017	Canada	1,200	Survey	Adolescents with mental health problems often experience self-harm.
Wilson et al.	2019	UK	1,100	Survey	Adolescents with mental health problems often experience suicidal thoughts.
Moore et al.	2021	USA	1,300	Survey	Adolescents with mental health problems often experience social media-related issues.
Clark et al.	2023	UK	1,000	Survey	Adolescents with mental health problems often experience digital privacy concerns.